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# Bio-Crosslinking of Starch Films with Oxidized Sucrose

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**BIO-CROSSLINKING OF STARCH FILMS WITH OXIDIZED SUCROSE**

by

Hazal Canisag

**A THESIS**

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# CROSSLINKING OF STARCH FILMS WITH OXIDIZED SUCROSE

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In this work, corn starch films were crosslinked with a novel bio-based agent; oxidized sucrose and their strength and stability were improved. Although starch is an inexpensive, biodegradable and abundant source, industrial applications of starch films are limited due to their poor mechanical properties. Crosslinking is one of the most common methods to enhance the mechanical properties of starch films. However, most of the available crosslinkers, such as glutaraldehyde, phosphorus oxychloride, sodium trimetaphosphate, and epichlorohydrin, are either toxic, expensive or with low crosslinking efficiencies. Oxidized sucrose, which is derived by periodate cleavage, has aldehyde groups in the structure and this enables it to be used as a crosslinking agent for starch films. Mechanical properties of crosslinked films and non-crosslinked films, such as tensile strength and elongation, were studied to verify the crosslinking effects of oxidized sucrose. The highest tensile strength obtained in this research is 22.9 MPa with 59.5 % elongation. The chemical and molecular structures of the films were also examined using Fourier Transform Infrared spectroscopy (FTIR),  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR and Differential scanning calorimetry (DSC). The water stability and formic acid stability of starch films were studied. The results show that crosslinking increased the stability of corn starch films both in water and formic acid. Last but not least, kinetic study of bio-

crosslinked corn starch films was studied via iodometric titration and crosslinking reaction rate constant, order of reaction and activation energy were calculated.

*Dedicated to my parents, Doruk Ozturk, and my faithful friends*

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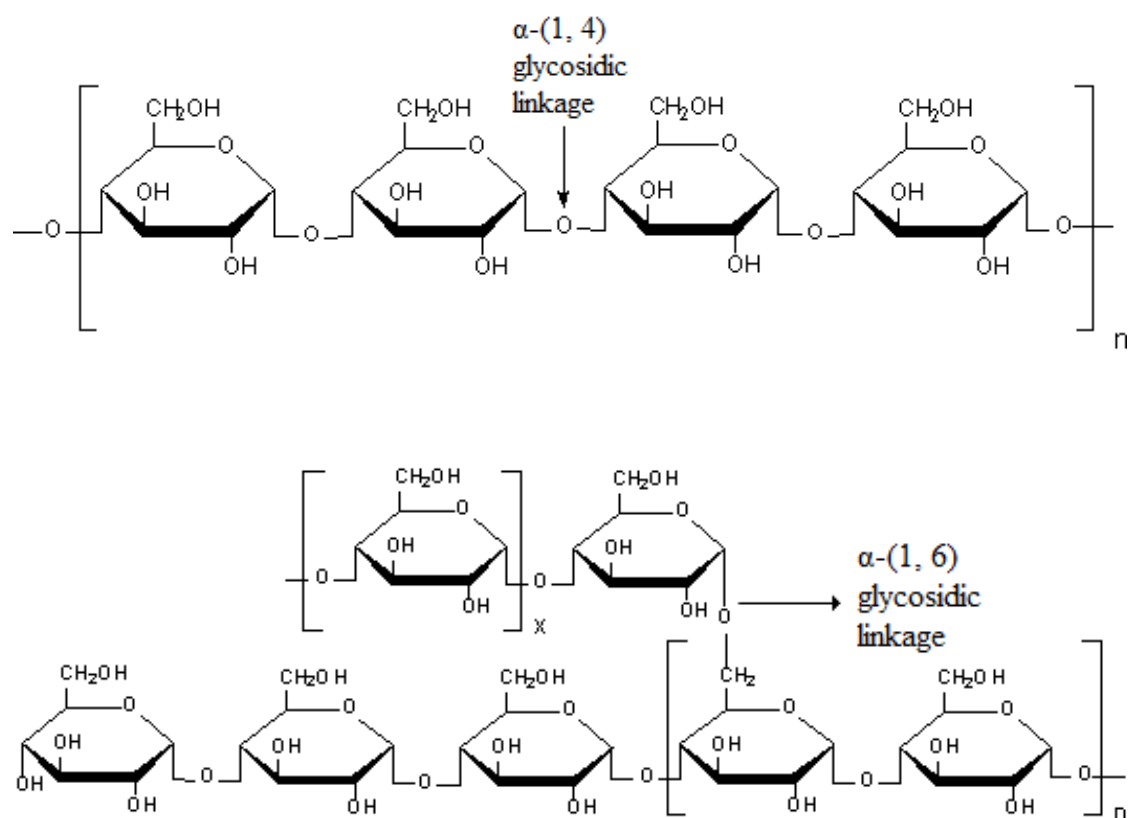


Figure 2. Structure of Amylose (above) and Amylopectin (below)

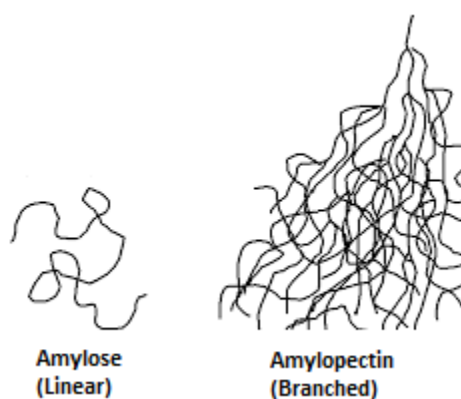


Figure 3. Linear and branched polymers, respectively

As a primary energy reserve, starch exists in plant roots, bulbs, seeds and stalks. It can be found in potato, corn, rice, wheat, cassava and etc. The size, shape, structure and chemical composition of starch granules vary depending on the origin of the starch. Depending on the source of starch granules, there are normally 15-30% amylose and 70-80% amylopectin in starch.

Native starch granules consist of amorphous and semi-crystalline regions due to the amylose and amylopectin arrangements. It is believed that amylose units exist in the amorphous non-crystalline regions of starch, while clustered organization of amylopectin chains form the crystalline regions (Putaux 2003), and these regions are important for the starch modification.

Starch granules are not soluble in cold water due to the strong hydrogen bonds between starch chains. However, when starch is heated in water, the crystalline structure is disrupted and water molecules can interact with the hydroxyl groups (-OH).

Starch is a renewable material and it is non-toxic and biodegradable (Lourdin et al. 1995). In addition to these, starch is an inexpensive polysaccharide. Starch is used in cooking to thicken food such as sauces or pudding. It is also used as emulsifier, stabilizer and fat replacer packaging in food applications. In addition to its use as a food additive, it is used in edible or biodegradable coatings and food packaging materials. Beyond its use in food industry, starch has been used in many other non-food applications, such as pharmaceutical industry, papermaking and textiles. It is reported that about 55% of total starch is used for food applications and 45% is for non-food applications (Myllarinen, 2002).

Since starch has an excellent film forming capacity, starch films have been used for many decades, especially as sizing agents in textiles and paper industries. They can be obtained from the native starch by casting, thermoplastic processing and etc. These biodegradable films provide an alternative material for plenty of industries that mentioned earlier. Mainly due to the environmental issues, petroleum based materials are being replaced by natural polymers, and starch is one of the most common polymers for that purpose. However, films produced from native starch has some disadvantages that should be reduced by modification.

### **1.1.1 Starch as a Sizing Agent**

Sizing or Size is a substance that is applied to or incorporated in other materials, to protect the material or to improve some properties of the material. Sizing is used especially in papermaking and textile manufacturing.

In papermaking, the main function of sizing is to increase the printability of the paper. It increased the resistance to penetration of liquids in the paper and this behavior allows inks or dyes to remain and dry on the surface of the paper, rather than be absorbed into the paper. This provides an economical solution to paint, print or write on the papers. There are two main types of sizing; surface sizing and internal sizing. Internal sizing agents must be strongly bonded with the cellulosic fibers of the paper. On the other hand surface sizing agents form a thin film on the paper and it joins with the fiber by the hydrophilic ends, , whereas hydrophobic ends facing outwards. This thin film increases surface strength, printability and water resistance of the paper (Robert Henderson, 1947).

In textile manufacturing, except for filament yarns, most warp yarns need to be sized to get protected from the abrasive forces (Schutz, 1977). It reduces the breakage of the yarn and again increases the strength of the fiber. In addition to the improved strength, sizing decreases the hairiness of yarns. The type of yarn material and the thickness of the yarn will determine the sizing recipe. Different from paper sizing, textile sizing requires a desizing step for the woven fabrics. Removing sizing agents is important to prevent the further effects of the chemicals.

There are different natural and synthetic chemicals used as surface sizing agents such as modified starches, glue, albumen, gelatin, carboxymethylcellulose (CMC), polyvinyl alcohol (PVA), etc. Due to the environmental problems, there has been a growing interest in biodegradable sizing agents for many decades.

### **1.1.2 Disadvantages of Starch-based Materials**

There has been a growing interest in the use of biodegradable polymers in order to reduce the environmental problems caused by plastic wastes. Among different kinds of biodegradable polymers, starch has been studied for this purpose. In addition to being abundant, renewable, biodegradable and inexpensive, starch has an excellent film forming capacity. Use of starch for biodegradable films has generated much interest in textile, packaging and paper industries for many years. Natural starch and its derivatives are almost 75% of the sizing agents used in the textile industry (Li and Zheng, 2008). However, even if their film forming capacity is excellent, the pure starch films have some disadvantages.



First of all, native starch contains a large number of hydroxyl groups, which are hydrophilic. Normally, starch granules are not soluble in cold water because of the fact that strong hydrogen bonds hold the starch chains together. However, when starch is heated in water, the crystalline structure is broken and water molecules will be able to interact with the hydroxyl groups this time. This causes the partial solubilization of starch granules and starch films. Thus, this hydrophilic nature of starch causes low water stability and high moisture sensitivity, which limit the development of starch-based materials.

Secondly, because of the amorphous regions in the structure, which are formed by amylose and the branching points of amylopectin, starch has low mechanical properties, such as low strength, low elongation and brittleness. Thus, high speed weaving processes cannot be used for the yarns sized with native starch. Several approaches have been studied to overcome these drawbacks and to make the starch-based products more convenient for industrial applications.

### **1.1.3 Starch Modification Methods**

Modification of starch is carried out to eliminate the shortcomings of the native starch mentioned earlier, to increase its versatility and to satisfy consumer demand. Numerous methods have been developed to produce modified starches with different characteristics for different applications. Starch modification methods can be broadly classified into five categories; physical, chemical, grafting, enzymatic and genetical modifications (Bemiller, 1997).

Physical modifications of starch are mainly used to change the granular structure of starch and to increase the cold water solubility. They do not involve any chemical treatment and they are cheap and simple. Heat moisture treatment, annealing, retrogradation, freezing, ultra-high pressure treatment and gelatinization are some of the common physical modifications of native starch (Light, 1989). However, since these kinds of modifications do not provide any chemical changes, they cannot be applied to increase the film forming capacity.

Genetical modifications and enzymatic modifications are generally used for the food and pharmaceutical industries. They involve producing high-amylose starch granules.

On the other hand, chemical modification, which involves etherification, esterification, oxidation, hydrolysis and crosslinking, is the most common method to improve the film forming capacity of starch. This method introduces new functional groups into the starch molecule and changes the physicochemical properties of starch. The chemical modification of starch changes the three available hydroxyl groups (C2, C3 and C6). Chemical modifications facilitate intra- or inter-molecular bonds at random positions in the starch structure. Etherification and esterification include substitution of hydrophilic hydroxyl groups of starch. The modified starch by etherification or esterification has newly formed carboxyl groups, such as acetyl and the properties of modified starch vary depending on these groups. Oxidation of starch is produced by reacting starch with an oxidizing agent and this method introduces new carbonyl and carboxyl groups to the starch structure. Hydrolysis of starch is generally done with an appropriate acid, such as hydrochloric acid, nitric acid, sulfuric acid and etc. Crosslinking is another common chemical modification method, which is done by utilizing various

crosslinking agents into the starch structure. These agents reinforce the hydrogen binds with chemical bonds and improve the properties of native starch. It alters not only the physical properties but also the thermal characteristics of starch.

In addition to all these, modification of starch can also be achieved by irradiation without any chemical additives which is called grafting.

## **1.2 Crosslinking**

A crosslink is a covalent or ionic bond that connects one polymer chain to another, and makes the polymer strong. Crosslinking is the formation of these bonding bridges between adjacent molecules by using different chemicals, which are called crosslinking agents. In other words, crosslinking means the polymer molecules are intra and interconnected by some sort of bonding.

In order to crosslink polymers containing hydroxyl groups, such as starch, the crosslinking agent must be able to react with at least two hydroxyl groups in a single polymer molecule (Steiger, 1966).

Crosslinking is one of the most commonly used approaches to improve the mechanical properties and water stability of starch films. For starch crosslinking, it occurs when a bifunctional or multifunctional crosslinking agent forms intermolecular linkages with the primary hydroxyl groups ( $C_6\text{-OH}$ ) or the secondary hydroxyl groups ( $C_2\text{-OH}$  and  $C_3\text{-OH}$ ). Thus, crosslinking increase intermolecular interactions between starch chains as seen in figure 3 and tie the molecules together.

Starch and starch products have been crosslinked with numerous crosslinking agents, such as phosphorous oxychloride, sodium trimetaphosphate (STMP), sodium

tripolyphosphate (STPP), epichlorohydrin, formaldehyde, boric acid, etc. Crosslinking agent concentration, starch content, pH, treatment temperature and treatment time changes the extent of crosslinking. These reaction conditions vary depending on the crosslinking agents used.

Crosslinked starches have superior features compared to native starches, such as high mechanical and thermal properties, high water stability, resistance to high temperature and low pH, due to the stronger structure.

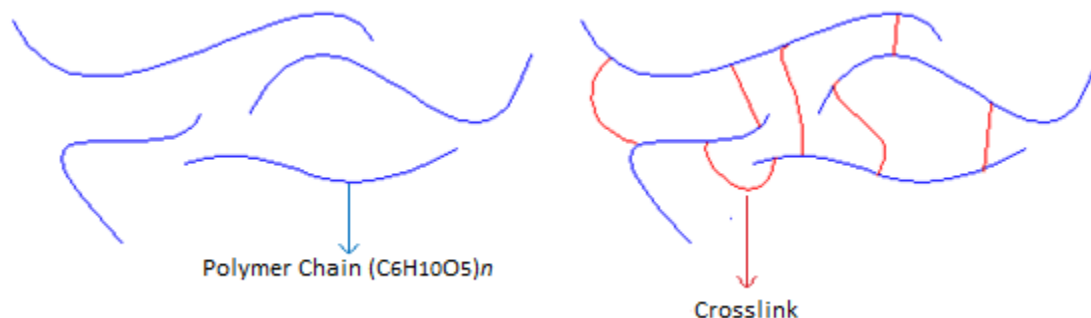


Figure 4. Native starch and crosslinked starch structure, respectively

### 1.3 Plasticizer

Plasticizer is an additive that increases the plasticity of the material applied to.

Since native starch films are brittle due to the numerous hydroxyl groups in the structure, another additive, plasticizer, should be used while crosslinking occurs. Plasticizers interrupt hydrogen bonding along the polymer chains as seen in Figure 5. The plasticizer molecules are very much smaller than the chain molecules of starch and they are able to enter between starch macromolecules. Then they set the polymer

macromolecules apart from one another and allow them to slide past one another. Thus, adding plasticizer creates a loose, freely mobile structure and makes the starch films flexible. When plasticizers are used with crosslinking agents, the starch films will have strong crosslinks with less hydroxyl group, which provide them higher strength with higher elongation. Thus, plasticizer addition overcomes film brittleness caused by high intermolecular forces and provides flexibility to the starch films.

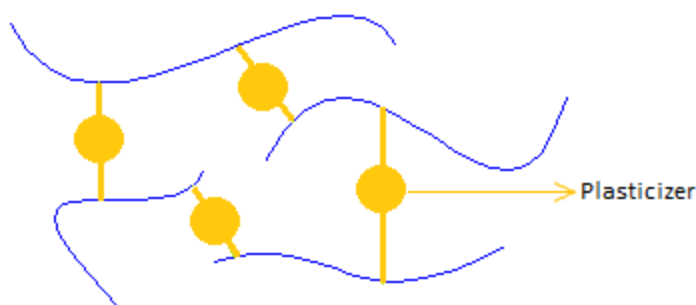


Figure 5. Native starch with plasticizer

For the starch films, the most commonly used plasticizer is polyols, such as glycerol, ethylene glycol, sorbitol and etc.

#### 1.4 Sucrose

Sucrose is a disaccharide that is also called ordinary table sugar. It is the most widely occurring disaccharide and is found in all photosynthetic plants. The structure of sucrose resembles the structure of starch. It also glucose repeating units like starch. However, it also has another repeating unit, fructose, which is linked with glucose unit via an ether

bond between C1 on the glucose and C2 of fructose as seen in Figure 6. This bond is called a glycosidic linkage again. Since sucrose does not have any anomeric hydroxyl groups (which is indicated as the hydroxyl group that is linked to the anomeric carbon, which is bonded to two oxygens in a ring structure), it is classified as a non-reducing sugar. In other words, sucrose does not contain hemiacetal or hemiketal groups like reducing sugars. It only has acetal and ketal groups

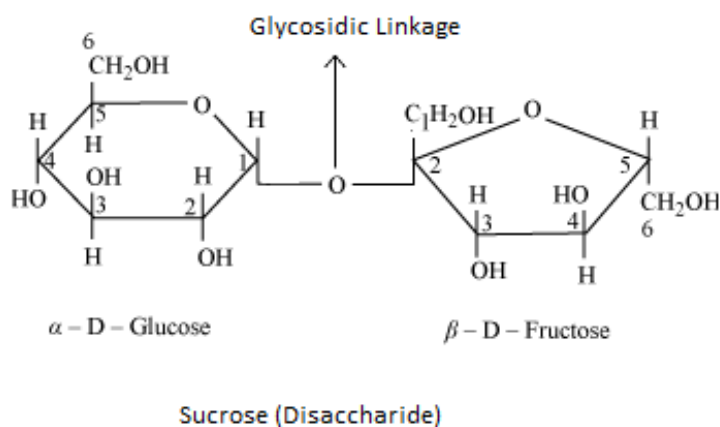


Figure 6. Repeating unit of Sucrose

## **CHAPTER 2: OBJECTIVES**

The main objective of this research is to improve the mechanical properties and water stability of corn starch films by crosslinking with a green agent. The goal is to use a novel crosslinking agent, oxidized sucrose, to obtain corn starch films with better tensile strength, better elongation and higher water stability both in dry and wet states. This study aims to eliminate the use of synthetic polymers for starch films by introducing this new green crosslinking agent. Last but not least, kinetics study has been done to determine the crosslinking rate and the reaction order.

## CHAPTER 3: LITERATURE REVIEW

### 3.1 Limitations of Available Crosslinkers

Although there is a growing interest in crosslinking starch, there have been limited studies with pure starch films. Crosslinking high amylose starch films has been done with epichlorohydrin (EPI) and tensile strength improvements were reported (Rioux et. al., 2002). Additionally, it has been used for banana starch crosslinking and decrease in dissolution was reported (Garcia et. al., 2009). However, epichlorohydrin is classified by several international health research agencies and groups as a probable human carcinogen compound. Phosphorous oxychloride has been used for crosslinking banana starch by the same authors, however no increase in strength and elongation were reported.

Citric acid, which is also a green chemical, has been used for corn starch crosslinking (Reddy and Yang, 2009). It has been reported that tensile strength, elongation at break, water stability and acid stability increased after crosslinking. Although citric acid is an environmentally friendly compound, the crosslinking reaction needs sodium hypophosphite (50% on weight of crosslinker used) as a catalyst, which is reported to be hazardous to the environment.

On the other hand, blending starch films with synthetic polymers is another common approach as a starch modification method. Starch-polyvinyl alcohol blended films crosslinked with hexamethoxymethylmelamine had appreciable improvements in tensile strength and elongation (Chen et. al., 1997). However, higher elongation was reported



only at high relative humidity with big amount of PVA and the whole process is expensive.

Potato starch-polyethylene blended films crosslinked with epichlorohydrin showed increase in tensile strength and elongation (Kim et. al., 2002). However, polyethylene is a synthetic polymer, which biodegrades slowly and epichlorohydrin is a carcinogen compound as mentioned above.

Similarly, starch-polyethylene blended films were crosslinked with boric acid (Yin et. al., 2005). However, decrease in elongation after crosslinking was reported and tensile strength did not show a reasonable change, even if the synthetic polymer was blended with starch.

Starch-polyvinyl alcohol crosslinking with formaldehyde and epichlorohydrin were reported (Das et. al., 2010). Both crosslinkers increased the mechanical properties. However, formaldehyde and epichlorohydrin are two of the most harmful crosslinkers and they cannot produce a biodegradable starch film.

Starch and polyvinyl alcohol were blended by reactive blending with a coupling agent and appreciable improvements were reported in terms of strength and elongation (Chang, 2000). However, the two step process costs relatively more than the other methods. Also, polyvinyl alcohol is a synthetic polymer that has high price and sourcing difficulties.

As mentioned above, current crosslinking agents are either toxic or expensive. Most of them cannot be used without a synthetic polymer. Poly (carboxylic acids) can be considered as a green crosslinking agent. However, there are no studies to reduce the use of catalyst, which makes the crosslinking process expensive. Thus, we believe oxidized

sucrose crosslinking will bring a better alternative to make a biodegradable starch film with high mechanical strength and low cost among all the other crosslinkers.

## CHAPTER 4: MATERIALS AND METHODS

### 4.1 General Research Design

The general research design is shown in Figure 7. All films were obtained by casting.

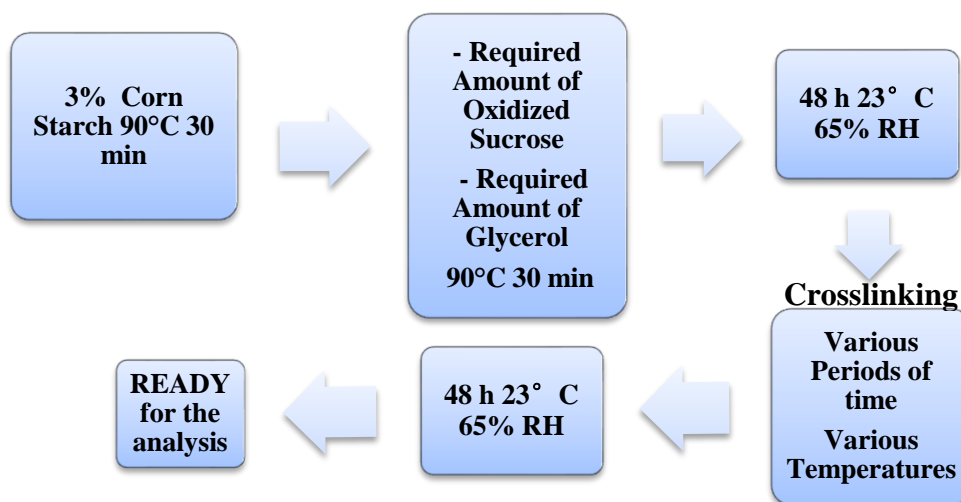


Figure 7. Preparation of Starch Films

### 4.2 Materials

Corn starch was purchased from Cargill Minneapolis, MN. Formic acid, which used for the acid stability test and sulfuric acid, which used for the kinetic study were purchased from EMD Chemicals Inc. with the purities greater than 98%. Formic acid is corrosive and skin or eye contact with formic acid or concentrated vapors cause severe chemical burns and damages. Sucrose and sodium periodate were used for oxidized

sucrose production and they were purchased from Johnson Matthey Company, Ward Hill, MA with the purities greater than 98% and barium dichloride. All the other chemicals used in this research were purchased from VWR International.

## **4.3 Methods**

### **4.3.1 Preparation of Oxidized Sucrose**

Oxidized sucrose was obtained by periodate cleavage. A mixture of sucrose (6.6 g), sodium periodate (12.9 g) and water (200 ml) was stirred at room temperature for 26 hours (Barry and Mitchell, 1954). Barium dichloride (7 g) was added to the mixture and the mixture was stirred at 5°C for 1 h to allow complete precipitation. After filtration of the mixture, the filtrate containing the polyaldehyde derivative of sucrose was stored at 5°C for the further use.

### **4.3.2 Preparation of Starch Films**

Starch films were prepared by 3% (w/w) starch in distilled water. The starch solution was heated on a hot plate to 90°C and held at that temperature for 30 minutes. For control films, pure starch solution and starch solution with 15% glycerol (on weight of starch used) were poured onto Teflon-coated plates. For the preparation of crosslinked films, required amounts of oxidized sucrose (1%, 3%, 5%, 8%, 10%, and 15%) were dissolved in the starch solution. The solution was heated to 90°C and held at that temperature for 30 minutes. Then, required amounts of glycerol (5%, 10%, 15%, 20%, 30% and 40%) were added to the solution. The solution was poured onto Teflon-coated plates. The cast films

were allowed to air dry for 72 hours and later peeled from the plates. The starch films were then treated in an air oven at various temperatures (140, 145, 150, 155, 160, 165°C) for various periods of time (3, 5, 8, 10 and 15 minutes). After heating, the films were immediately transferred to the conditioning room at 23°C, 65% relative humidity.

#### **4.3.3 Characterization of Starch Films and Crosslinkers**

FTIR spectra were collected from sucrose and oxidized sucrose on an attenuated total reflectance ATR spectrophotometer (Nicolet 380: Thermo-Fisher, Waltham, MA) to see the structural difference after periodate cleavage. The samples were placed on a germanium plate and 128 scans were collected for each sample.

<sup>1</sup>H-NMR analyses were done to see the structural differences of sucrose / oxidized sucrose and non-crosslinked films / crosslinked films. For oxidized sucrose sample, the solvent was evaporated and the resulting sample was dissolved in D<sub>2</sub>O. The starch samples were dried and were dissolved in DMSO. Spectra were taken on a Bruker AVANCE DRX 500 MHz NMR spectrometer.

<sup>13</sup>C-NMR analyses were done to support the idea that crosslinking occurred between oxidized sucrose and starch. The starch samples were dried and dissolved in DMSO again. Spectra were taken on Bruker AVANCE DRX 500 MHz NMR spectrometer.

#### **4.3.4 Mechanical Properties of Starch Films**

The mechanical properties of starch films were determined on an MTS tensile tester according to ASTM Standard D 882-02. Testing was done on 5 samples each randomly cut from the cast films. Before testing, all samples were conditioned for at least 48 hours in a conditioning chamber maintained at  $23 \pm 0.5^{\circ}\text{C}$  and  $65 \pm 1\%$  relative humidity. Wet strength of the films was determined after conditioning the films at  $21^{\circ}\text{C}$  and 90% relative humidity for 48 hours.

Tensile strength, elongation (%), glycerol effect, oxidized sucrose effect, time effect and temperature effect on crosslinking were studied.

#### **4.3.5 Water Stability**

The control and crosslinked starch films were weighed about 0.3 g and put in 100 ml distilled water in room temperature. They were taken out after a specified time (3, 5, 10, 20, 35 days). The samples taken out were rinsed in distilled water and allowed to dry at  $23 \pm 0.5^{\circ}\text{C}$  in the oven. The weight of the conditioned sample after staying in water was used to determine the % weight loss of the starch films in water. Three replications were done for each specified time.

In addition to the water stability of corn starch films in room temperature, temperature effect on water stability was studied. About 0.3 g control and crosslinked films were weighed and put in 100 ml water in different temperatures (50, 60, 70, 80,  $90^{\circ}\text{C}$ ) for 30 minutes. The samples were taken out, rinsed with distilled water and

allowed to dry at  $23 \pm 0.5^{\circ}\text{C}$  in the oven. The weight of the conditioned sample after staying in water was used to determine the % weight loss of the starch films in water in different temperatures. Three replications were done for each specified temperature.

#### **4.3.6 Formic Acid Stability**

The weight loss of the control and crosslinked corn starch films in formic acid was studied in room temperature. Precisely 0.2 g of the films (control film and crosslinked films with different amount of sucrose) were put in 5 g formic acid and sealed tightly. They were kept at room temperature for 24 hours. After treatment, formic acid was removed and the remaining starch films were washed with distilled water couple of times. The weight of the dry sample after rinsing was used to determine the % weight loss of the starch films in formic acid. Three replications were done for each film.

#### **4.3.7 Thermal Analysis**

A differential scanning calorimeter (DSC 822: Mettler-Toledo Inc., Columbus, OH) was used to study the thermal behavior of the starch films before and after crosslinking. DSC curves were obtained by heating the samples at a rate of  $20^{\circ}\text{C}$  per minute and holding at  $50^{\circ}\text{C}$  for 3 minutes to evaporate moisture, and heating to  $250^{\circ}\text{C}$  at  $20^{\circ}\text{C}$  per minute. The samples were cooled to  $25^{\circ}\text{C}$  slowly and a second heating cycle was obtained by the same procedure.

#### 4.3.8 Reaction Kinetics

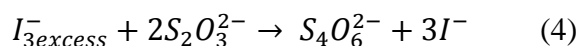
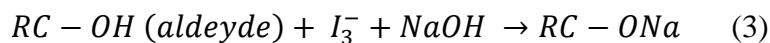
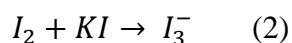
The goal of the kinetic study was to determine the rate law of oxidized sucrose crosslinking of starch films. After determining the rate order and the rate constant ( $k$ ), Arrhenius constant ( $A$ ) and the activation energy ( $E_a$ ) were calculated according to the Arrhenius Equation as seen in equation (1), where  $R$  is the gas constant, 8.3145 J/(molK) and  $T$  is the curing temperatures (K).

$$k = Ae^{-\frac{E_a}{RT}} \quad (1)$$

The reaction between aldehyde groups of oxidized sucrose and hydroxyl groups of starch can introduce either intermolecular or intramolecular crosslinks and the exact amount of crosslinking obtained by these linkages is almost impossible to measure. Thus, the amount of crosslinks formed as a result of the crosslinking reaction was calculated on the basis of total multifunctional crosslinking agents introduced into the aqueous starch solution. 0.5 grams of crosslinked films, which were cured at different temperatures (140°C, 150°C and 165°C) for various amounts of time (0, 3, 5, 10, 15 minutes) were put into 100ml distilled water. After 10 hours, the films were dewatered and the filtrates with the unreacted oxidized sucrose were collected. Same films were leached in 100ml distilled water and dewatered couple of times. Aldehyde concentrations in the filtrates were quantified using iodometric titration method. The unreacted oxidized sucrose concentration was subtracted from the total oxidized sucrose introduced into the starch solution in the beginning and plotted against the curing time to find out the reaction rate and reaction constant.



Iodometric titration method was developed to determine the remaining aldehyde groups' concentration. Excess amount (25ml) of 0.1 M  $I_2/KI$  solution and 10ml, 1M NaOH solution were added slowly to the filtrate obtained as explained above. The mixture was held in the dark for 20 minutes to complete the reaction between Iodine and aldehyde groups of oxidized sucrose as seen in equation (2) and (3). Since the final solution of oxidized sucrose is a mixture of different polyaldehyde derivatives, the reaction ratio between triiodine and aldehyde was assumed 1:1 as seen in equation (3). After 20 minutes, 15ml, 0.5 M  $H_2SO_4$  was added into the mixture in a controlled manner to adjust pH and to prevent the formation of sodium iodate. The mixture was titrated with the standardized sodium thiosulfate solution (0.1 M). The whole procedure was repeated for all samples. Since we do not know the molecular weight of oxidized sucrose, it was estimated based on the average of the possible maximum weight (the weight of sucrose, 342.49 g/mol) and the possible minimum weight (the weight of oxidized sucrose with 4 aldehyde groups, 308.00 g/mol). The aldehyde concentrations were calculated based on the consumed thiosulfate volume as seen in equation (4).



#### 4.3.9 Statistics

To determine whether there is a significant difference on the starch films' structures before and after crosslinking, one way analysis of variance was employed to analyze the data. The sample sizes were equal for each test, the observations being tested are independent within and among the groups, the groups were normally distributed and variances were homogenous. The main objective of this study was to examine the properties of crosslinked starch films with different oxidized sucrose concentrations, various temperatures and various times, which means it was needed to examine all possible pairwise comparisons. Thus, Tukey's pairwise multiple comparison was chosen. The confidence interval was set at 95% and a p value less than 0.05 was considered to be a statistically significant difference. The data in the figures labeled with different numbers or characters indicate significant differences among the data tested. Standard errors are shown by the error bars in figures.

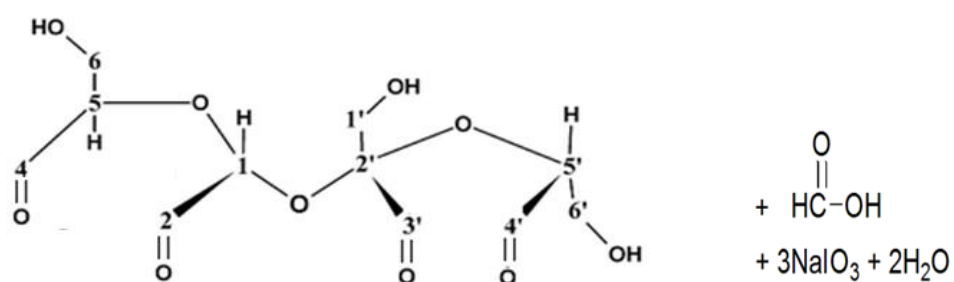
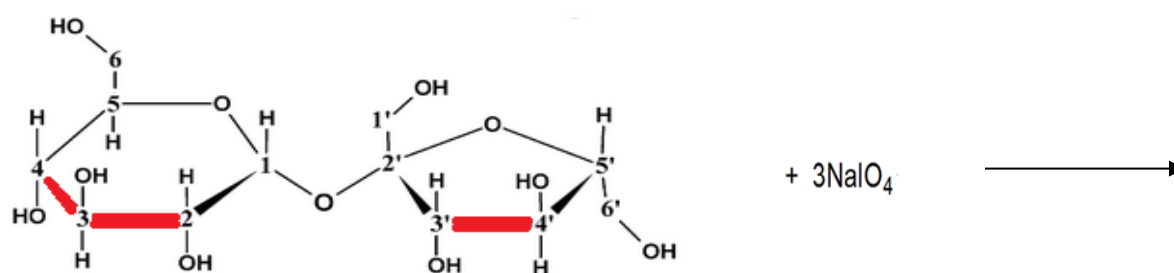
## CHAPTER 5: RESULTS AND DISCUSSION

### 5.1 Proposed Structure of Oxidized Sucrose and Characterization

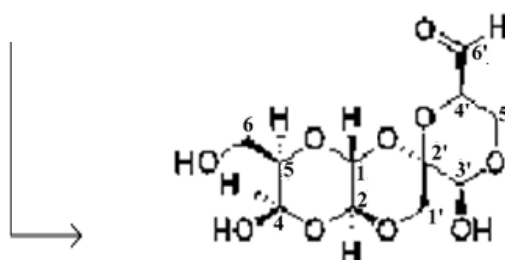
As it was stated earlier, sucrose is a non-reducing sugar and normally it does not generate compounds containing an aldehyde group. However, oxidized sucrose, which has aldehyde groups, was obtained with sodium periodate cleavage. Sodium periodate ( $\text{NaIO}_4$ ) was expected to break apart 1, 2-diols (vicinal diols) which are showed with red molecular bonds in Figure 8, to form polyaldehyde derivative of sucrose. Sodium periodate was reduced from Iodine 7 to Iodine 5 and two of the carbon-oxygen pi-bonds were formed.

One of the possible oxidation products can be seen in Figure 8. Additionally, the hemiacetal formation was expected to occur simultaneously, and three of the aldehyde groups were expected to react intermolecularly with the remaining hydroxyl groups. Therefore, the product of the oxidation was expected to be a mixture of hemiacetals and oxidized sucrose with aldehyde groups.

The newly formed oxidized sucrose has carbon double bonds and less hydroxyl groups. In Figure 8, it can be seen that C2, C3 and C4 of glucose unit and C3' and C4' of fructose unit of sucrose are expected to lose hydroxyl groups. Since FTIR and  $^1\text{H-NMR}$  spectra show difference due to the different molecular bonds in the chemical structure, the spectra of sucrose and oxidized sucrose are expected to be different according to the aldehyde groups and amount of hydroxyl groups.



One of the  
Tetraaldehyde  
Derivatives of  
Sucrose



One of the  
hemiacetal forms  
of Oxidized

Figure 8. Periodate oxidation of sucrose

In figure 9, FTIR spectra of sucrose and oxidized sucrose are shown. In the fingerprint region, which is between 600 and 900  $\text{cm}^{-1}$  and contains unique patterns for each different compound, C-O stretching modes of the glycosidic bond can be seen for both molecules. In both spectra, there is a band around 3400  $\text{cm}^{-1}$  which indicates the hydroxyl groups in the structure. Additionally, between 2900 and 2700  $\text{cm}^{-1}$ , there are C-H stretch bands in both spectra. On the other hand, evidence of the presence of aldehyde groups mentioned above can be found around 1650  $\text{cm}^{-1}$  in the oxidized sucrose spectra. This band is ascribed to the unsaturated aldehyde band. There should be another peak related to the aldehyde side group around 2700  $\text{cm}^{-1}$ , however it is lost in C-H stretching peak.

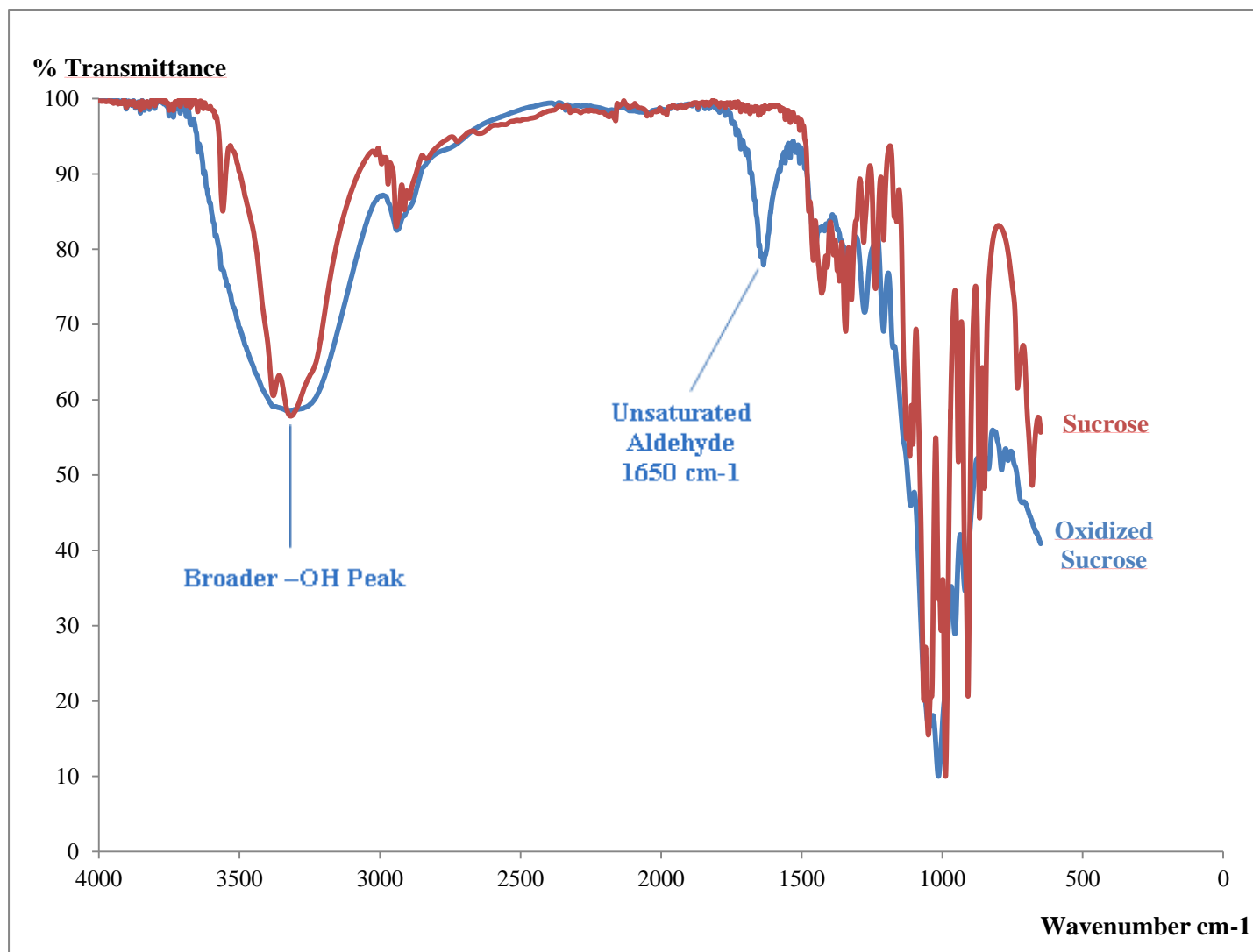


Figure 9. FTIR spectra of sucrose and oxidized sucrose

In Figure 10, the structural characterization of sucrose and oxidized sucrose by  $^1\text{H}$ -NMR are shown. Proton shift at 5.2ppm is made by alpha and beta stereoisomers of the glucose ring of sucrose. The same anomeric proton shift can also be seen at 5.1ppm in oxidized sucrose spectrum. Between 3ppm and 3.7ppm, unchangeable proton shifts are shown, which are the protons bonded with C5 and C6 in glucose unit and C1', C5' and C6' in fructose unit of sucrose (Figure 8 and Figure 10). Proton shift at 8.1 proves the aldehyde formation with periodate oxidation of sucrose. As seen in Figure 10, while pure sucrose does not have an aldehyde shift, oxidized sucrose has a sharp shift at 8.1ppm. Additionally, proton shifts at 5ppm indicate the intermolecular acetal protons due to the hemiacetal formation proposed earlier.

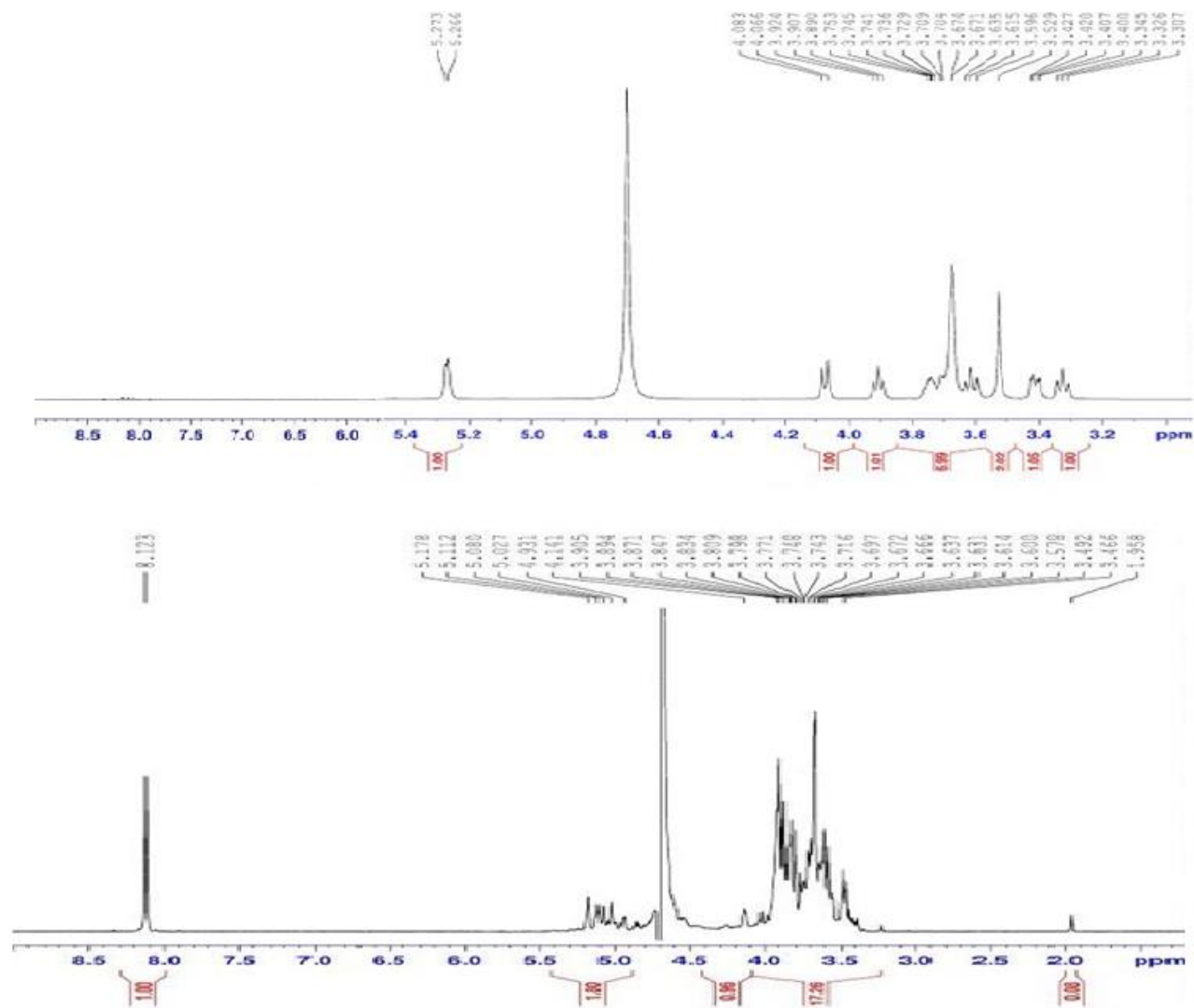


Figure 10.  $^1\text{H}$ -NMR spectrum of sucrose (above) and oxidized sucrose (below)



## 5.2 Proposed Crosslinking Mechanism of Starch Films and Characterization

Aldehyde groups of the oxidized sucrose are expected to chemically bond to hydroxyl groups in starch. The acetal formation is expected to occur by addition of alcohol to the aldehydes. Thus, after crosslinking, aldehyde groups in oxidized sucrose are expected to be lost and new C-O-C etheric bonds are expected to be formed in crosslinked corn starch films. The general organic reaction between oxidized sucrose and starch films, addition of alcohol to aldehyde, is shown in Figure 11.

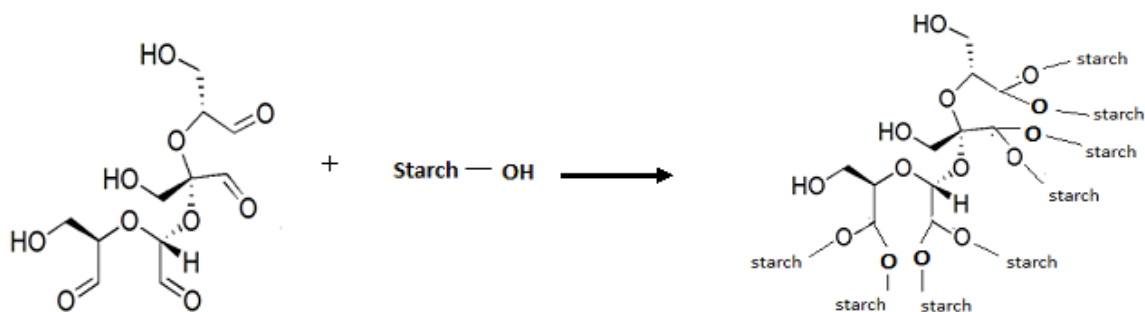


Figure 11. Starch crosslinking with oxidized sucrose

According to the acetal formation during crosslinking, the proton environment around C6 of the glucose ring, which is the reactive side for crosslinking, is expected to increase.  $^1\text{H}$ -NMR spectra of non-crosslinked and crosslinked films prove this phenomenon and the crosslinking as seen in Figure 12. The proton shifts around 5ppm show the hydrogen bonded hydroxyl groups. Since oxidized sucrose has also hydroxyl groups, this proton shift did not show a significant change after crosslinking. However, the proton shift at 4.6ppm is the evidence of crosslinking. The signal is splitting after crosslinking. Signal splitting in  $^1\text{H}$ -NMR occurs due to the changes in the proton environment. Thus, it can be easily said that this splitting in crosslinked films is due to the acetal bonds formed between C-6 of starch and aldehyde groups of oxidized sucrose, since it causes an increase around C6 of starch structure proposed earlier.

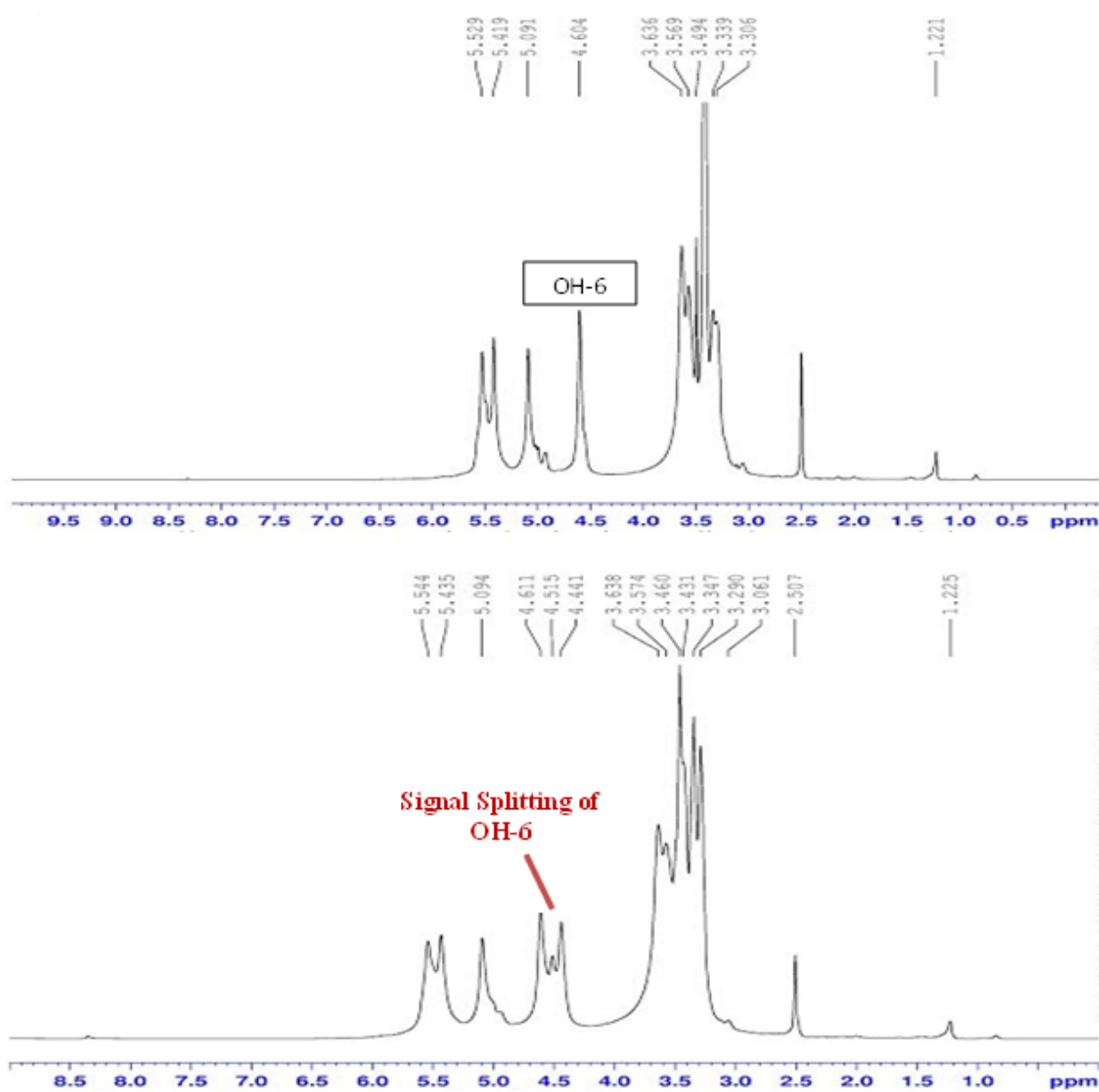


Figure 12.  $^1\text{H}$ -NMR spectra of non-crosslinked (above) and crosslinked (below) starch films

In Figure 13 and 14, the  $^{13}\text{C}$ -NMR spectra of non-crosslinked and crosslinked starch films can be seen. Both spectra have the anomeric C (C1) shift at 100.55ppm (non-crosslinked film) and 100.35ppm (crosslinked film). It is not expected the carbon environment around C1 to change after crosslinking. In 79.29ppm (non-crosslinked film) and in 79.11ppm (crosslinked film), C4 shifts can be seen. It is not expected any changes again around C4 environment. On the other hand, since C5 and C6 environment will change after crosslinking due to the C-O-C bond formation between starch and oxidized sucrose and it is expected to see a difference in these shifts in  $^{13}\text{C}$ -NMR spectra. As it was expected, the C6 (C-OH) shift in non-crosslinked film in 62.18ppm disappeared after crosslinking as seen in the magnified figure and sharp peaks around 65ppm and 75pm became unsharpened peaks, which are indicated by the red stars in Figure 13 and Figure 14.

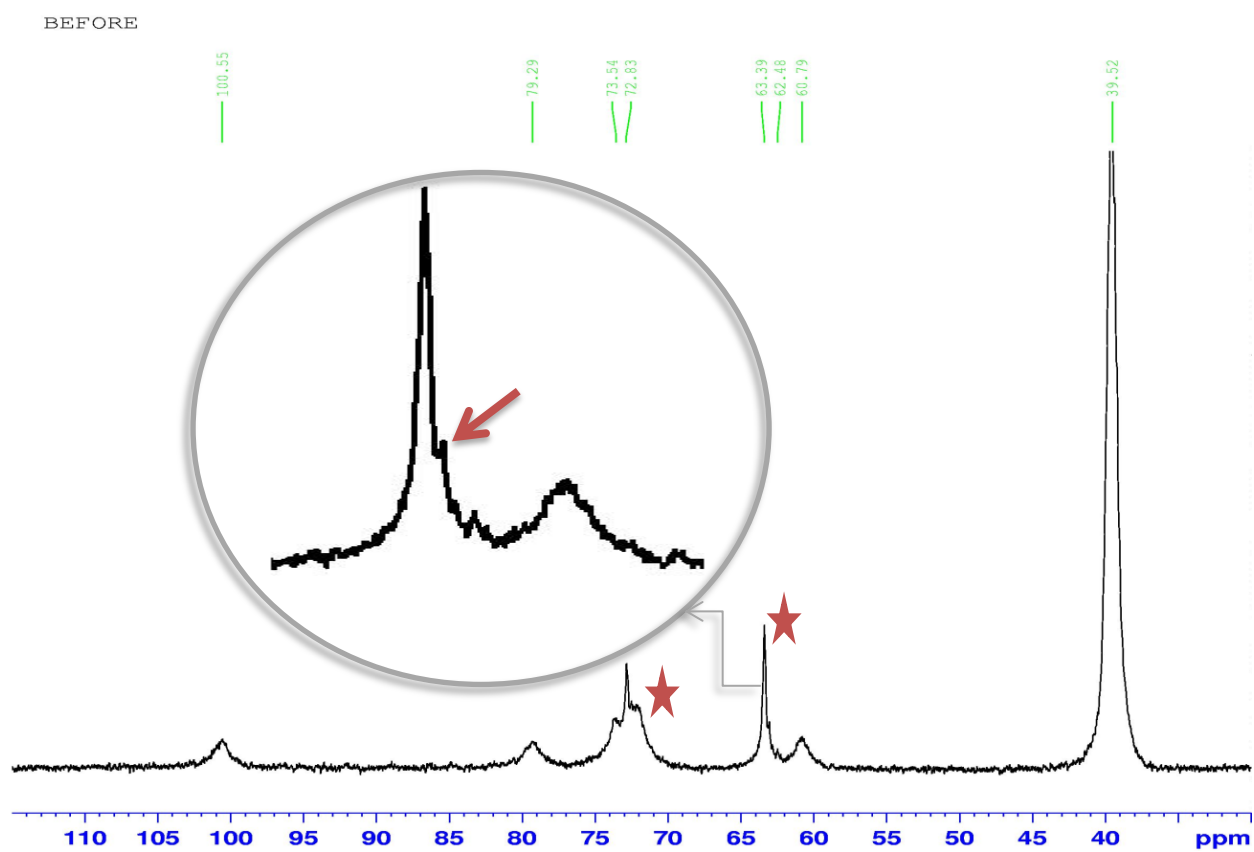


Figure 13.  $^{13}\text{C}$ -NMR spectra of non-crosslinked starch films

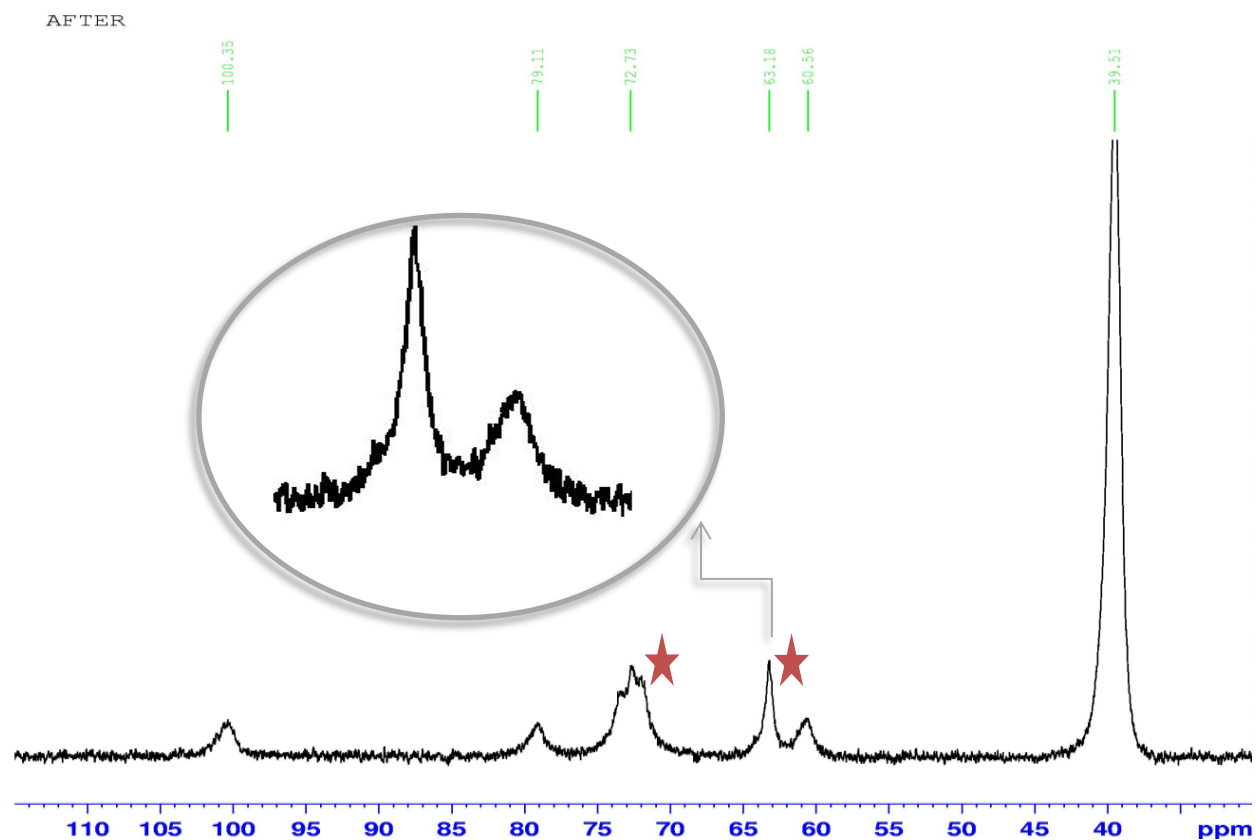


Figure 14.  $^{13}\text{C}$ -NMR spectra of crosslinked starch films

### **5.3 Crosslinking Effect on Mechanical Properties**

To see the crosslinking effects on mechanical properties of corn starch films, tensile strength and elongation were determined. Glycerol effect, oxidized sucrose effect, curing time effect and curing temperature effect on mechanical properties were studied.

#### **5.3.1 Effect of Glycerol Concentration**

Native starch films are brittle compared with synthetic polymers and they technically need to be plasticized. Starch is a very long, connected chains of atoms. When many starch chains are put together, they tend to entangle with each other and this makes it difficult for any individual chain to move easily. If energy is applied to a single starch chain, it tends to move in a particular direction. However, the other starch chains in that direction must move in order to the first chain to move.

Addition of plasticizers to polysaccharide system increases the flexibility of the films since plasticizer molecules interfere with polymeric chain association facilitating their slippage. The rigidity of the glycerol added starch films will have less rigid network and less ordered structure. Thus, the ability of polymer chain movements was expected to be increased. As one can see in Figure 15, addition of only glycerol to non-crosslinked starch films increased elongation due to the increased mobility and flexibility of the polymer chains. On the other hand, tensile strength showed a different fashion with increasing glycerol concentration. The glycerol molecules interrupt the polymer structure and decrease the intermolecular attractions between polymer chains. This causes not only higher mobility but also less resistance to breaking under tension. Tensile strength of

non-crosslinked starch films decreased with increasing glycerol concentration as it is seen in Figure 15. Non-crosslinked starch films with 3% glycerol give the highest tensile strength, 28.57 MPa, whereas films with 15% glycerol give the lowest tensile strength, 6.00 MPa.

When starch films are crosslinked with oxidized sucrose, there will be additional strong interactions between polymer chains in order to the hydrogen bonds. Thus, it will be harder for the starch chains to move when they are crosslinked. However, glycerol is a small molecule and it can interrupt the hydrogen bonds among the polymer chain. It will fit between the starch macromolecules and will cause them to separate slightly. This reduces the strong attraction between polymer chains, decreases the rigidity of the polymer network and increases flexibility. Since glycerol molecules are small and have low intermolecular interaction, they are able to move easily through the starch chains. The plasticizer can move aside with little energy, and the polymer chain can move into the plasticizer location with less energy. Because of that, elongation continues to increase with increasing glycerol concentration as one can see in Figure 16.

On the other hand, glycerol concentration has a different effect on tensile strength. There was no significant change in terms of tensile strength and elongation in lower concentrations, because glycerol did not provide enough plasticizing effect. It was not capable to break enough hydrogen bonds to make the films flexible. With increasing concentration, there will be more molecules to share the energy as mentioned above, so tensile strength will increase with increasing glycerol concentration. However, after a certain point, strength starts to decrease again. A high amount of glycerol starts to break more hydrogen bonds and there will be a big space between two chains. Even if the



polymer can move easily, since it cannot form the hydrogen bonding again, tensile strength starts to decrease again.

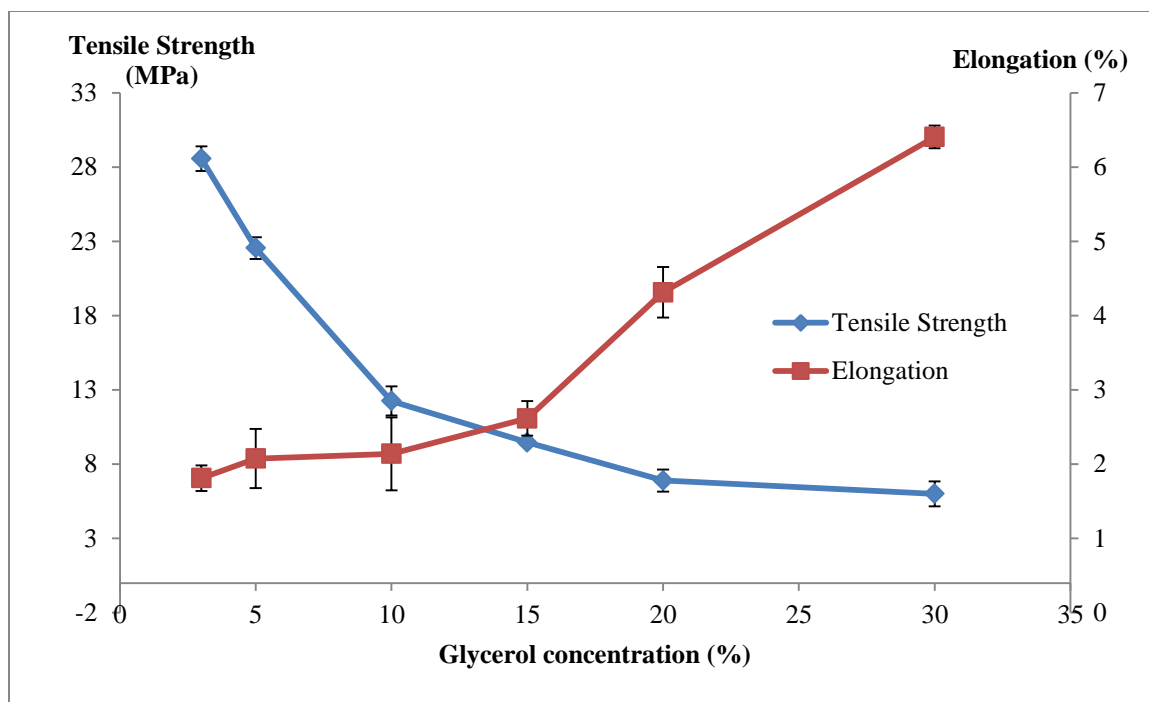


Figure 15. Glycerol concentration effect on tensile strength and elongation of non-crosslinked starch films

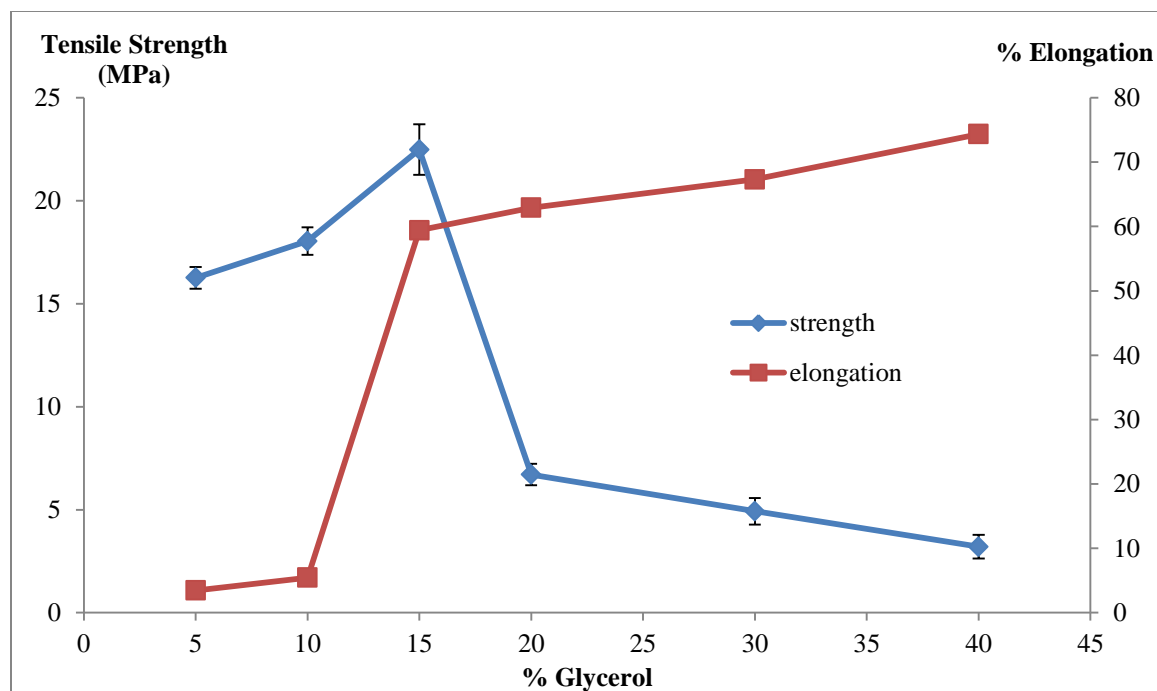


Figure 16. Glycerol concentration effect on tensile strength and elongation of 5% crosslinked starch films at 165°C for 5 minutes

### 5.3.2 Effect of Oxidized Sucrose Concentration

Crosslinking starch films with oxidized sucrose improved tensile strength of the films by more than 160%, as seen in Figure 16. Crosslinking builds strong intermolecular connections. However, there is an optimum amount of oxidized sucrose that is necessary to obtain good increase in both tensile strength and elongation of the films. Concentrations of oxidized sucrose less than 5% did not provide more improvements. At low concentrations, oxidized sucrose was not able to build enough intermolecular connections between starch chains and could not provide better tensile strength. On the other hand, with increasing oxidized sucrose concentration, tensile strength started to decrease after a certain point.

When crosslinking agent concentration was more than 5%, too much interconnection were built between starch chains. Because of that, when energy is applied the friction will increase and even if there are a lot of crosslinks, the films will be broken by the weak point. Additionally, elongation continued to increase until 10% oxidized sucrose. Thus, this shows us after a certain amount of oxidized sucrose, the interconnections between starch chains increased significantly and applied energy started to break the interconnections one by one. In other words, all starch chains were not broken apart and the whole structure was still elongating. This leads the films to lower tensile strength again. However, since there are still strong interconnections between two chains and the distance between chains is enough to move, elongation continues to increase. On the other hand, after 10% oxidized sucrose, both tensile strength and elongation decreased. This shows that excess crosslinking started when oxidized sucrose concentration is greater than 10%. When there are too many interconnections between starch chains, the

distance between these chains become lower and the chains cannot slip over each other.

Instead of this, they will start to rupture with applied energy

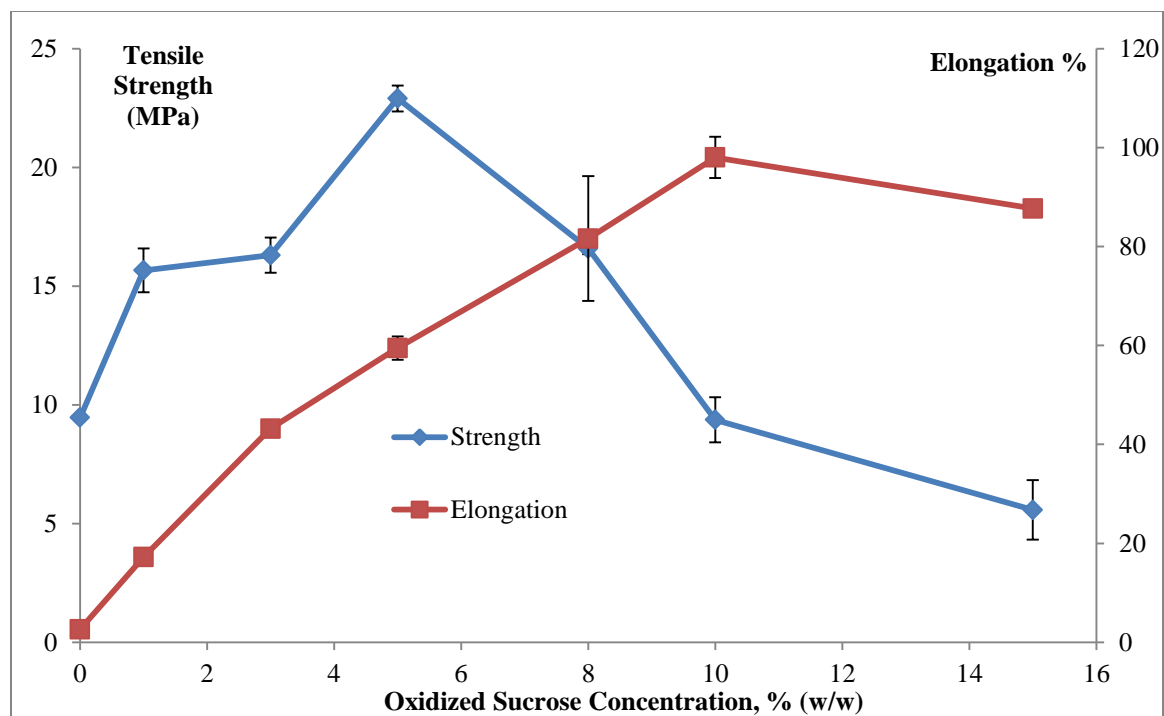


Figure 17. Effect of increasing oxidized sucrose on strength and elongation of crosslinked starch films with 15% glycerol, at 165°C for 5 minutes

### 5.3.3 Effect of Curing Time

Curing time is an important parameter for starch crosslinking, since starch molecules are sensitive to high temperatures and could be damaged. Corn starch crosslinking with oxidized sucrose were done with using various curing times as seen in Figure 17. Adequate curing time is a necessity for the crosslinking reaction to occur. However, excess curing will damage the starch molecules and lead to decrease in the mechanical properties. Also unwanted color changes may occur due to excess curing. Optimum tensile strength and elongation were achieved with 5 minutes curing. Further increase in curing time had negative effects on tensile strength. Curing temperature was chosen as 165°C to study the curing time effect. Holding corn starch films under high temperature for long periods of times could break the backbone of starch molecules and could damage them. Because of that, even if more crosslinks can be formed with increasing curing time, starch molecules will start to be damaged and this will decrease the tensile strength.

On the other hand, elongation showed an interesting fashion with the increasing curing time. After 5 minutes curing, elongation also started to decrease as tensile strength. This was mainly caused by crosslinking hindered molecular chain slippage. However, there is not a significant difference between 10 minutes curing and 15 minutes curing. Even increasing crosslinking will damage the backbone of starch and cause lower strength, since there will be plenty of crosslinks in the structure, elongation will not be affected anymore after 10 minutes. The corn starch films will still be able to stretch more than the non-crosslinked films.

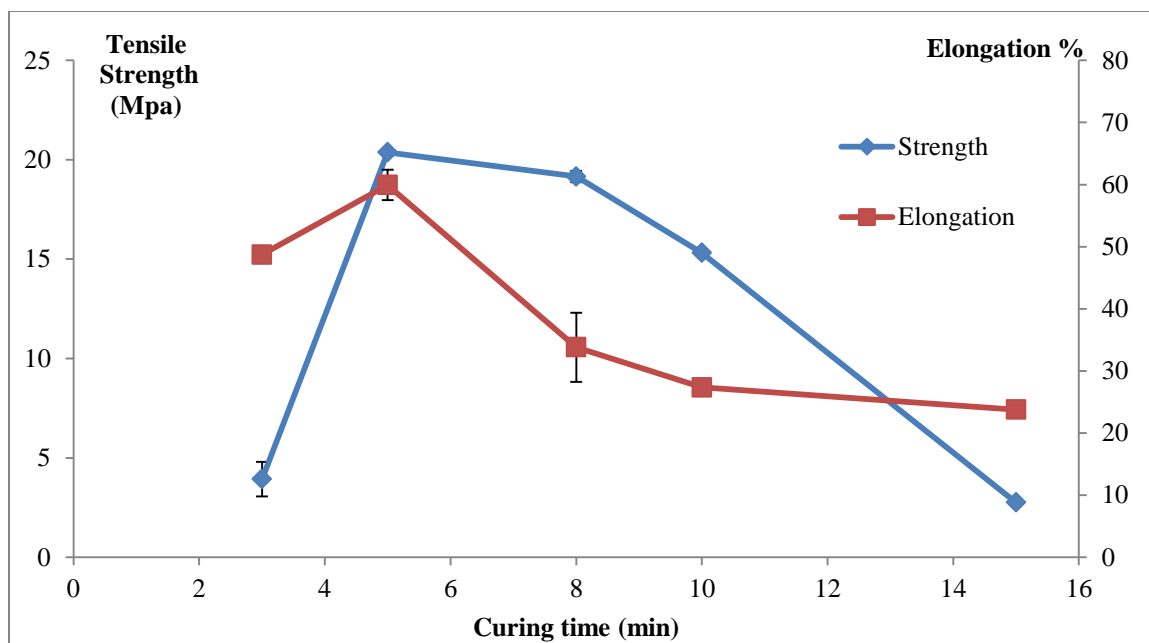


Figure 18. Effect of curing time on strength and elongation of crosslinked starch films with 5% oxidized sucrose and 15% glycerol, at 165°C

#### **5.4 Effect of Curing Temperature**

Since starch can easily decompose at high temperature, curing temperature is significant for crosslinking corn starch films. The temperature effect was studied and starch films cured at different temperatures (145-165°C). Tensile strength and elongation increased with increasing temperature and 165°C gave the highest elongation, 61.91% and highest tensile strength, 24.26% as seen in Figure 18. The results show that the crosslinking reaction between corn starch and oxidized sucrose was not completed below 165°C. Additionally, after 150°C, both tensile strength and elongation showed significant increase, which indicates the curing temperature should be more than 150°C to have a sufficient crosslinking reaction. Thus, the optimal curing temperature is 165°C. Since starch can be damaged easily by heat, further temperatures were not studied and 165°C was chosen as an optimal temperature.



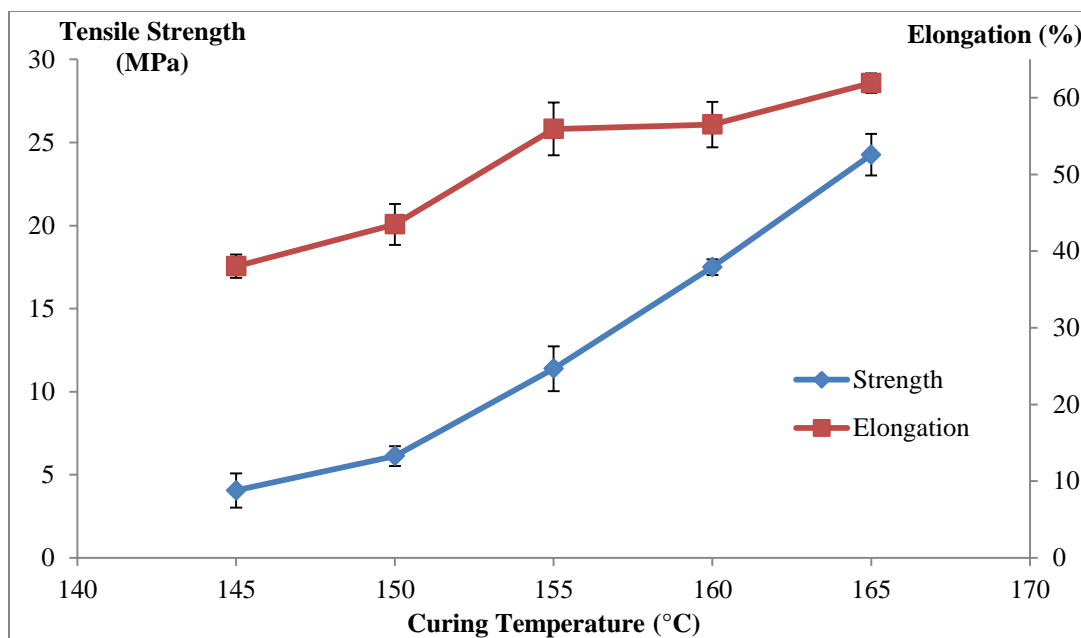


Figure 19. Effect of curing temperature on strength and elongation of crosslinked starch films with 5% oxidized sucrose and 15% glycerol for 5 minutes

## 5.5 Weight Loss in Water

As it was stated earlier, corn starch films are not soluble in cold water. However, their water stability in hot water is weak due to their hydrophilic nature and crosslinking is expected to increase their stability both in cold and hot water. After crosslinking starch with oxidized sucrose, water stability in water at room temperature increased as seen in Figure 19. After staying in water for three days, control films lost %25.3 their weight. On the other hand crosslinked weight loss was only 15.45. After ten days in water, the weight loss of crosslinked films slightly increased, 24%. Even after thirty five days in water, the weight loss of crosslinked films was 24%, whereas the non-crosslinked films lost about 80% of their weights.

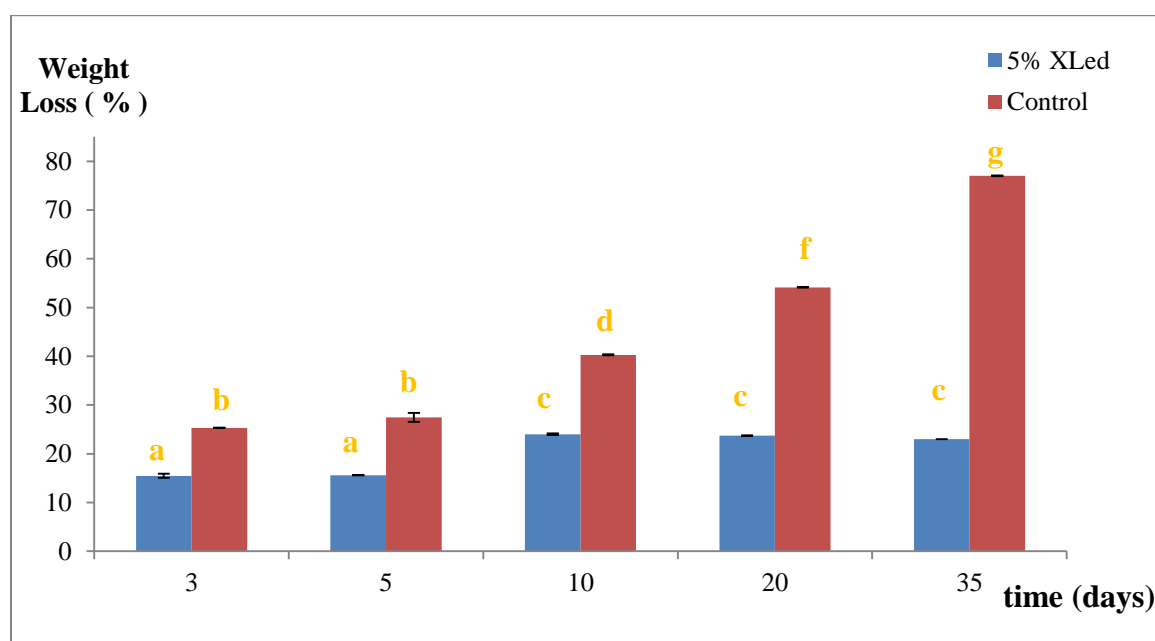


Figure 20. Weight loss of control and 5% crosslinked starch films at 25°C in distilled water. Both films were with 15% glycerol and the crosslinked films were cured at 165°C for 5 minutes.

In addition to the water stability in room temperature, weight loss of crosslinked corn starch films was measured as a function of temperature (50-90°C) as seen in Figure 20. Increasing temperature did not affect crosslinked corn starch films' stability in water. Even the films stayed at 90°C for 30 minutes, the weight loss was not more than 25.22%. Thus, all these results prove that crosslinking increases not only the strength and elongation of corn starch films, but also the stability of the films in cold and hot water. It decreases dissolution by creating three dimensional polymeric structures. This means the starch chains will not be linear anymore after crosslinking and the accessible regions in the structure will decrease. This fashion prevents swelling in water and interacting with water molecules. Thus, crosslinked starch films have better stability in water, even if they are heated in water.

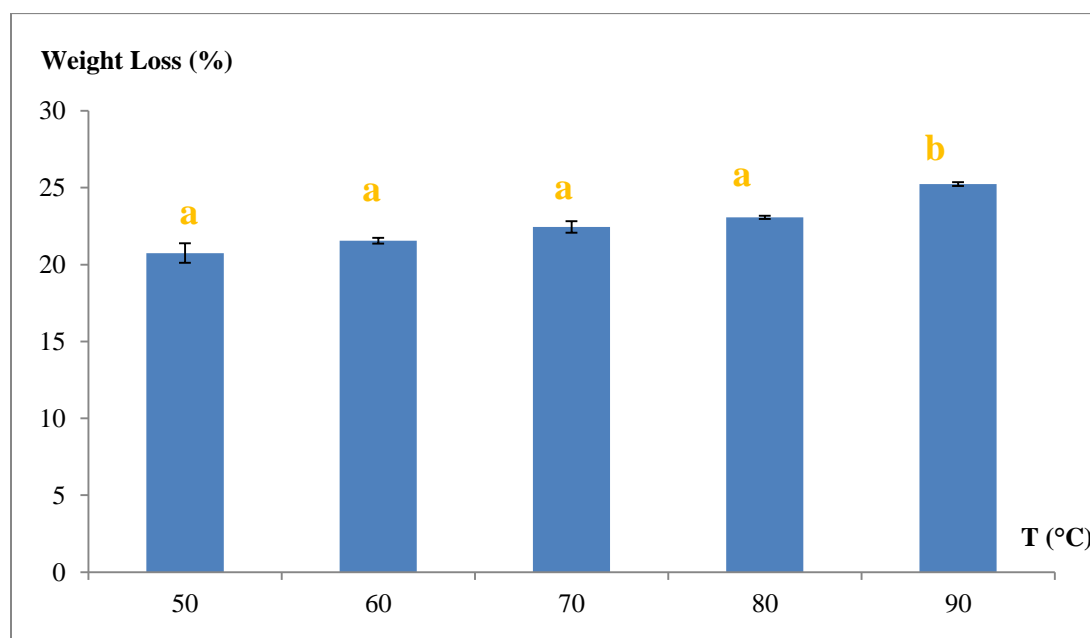


Figure 21. Temperature effect on weight loss of 5% cross-linked starch films in water. All films were with 15% glycerol, and cured at 165°C for 5 minutes.

## 5.6 Weight Loss in Formic Acid

Formic acid is the simplest carboxylic acid with the chemical formula of  $\text{HCOOH}$ . Native starch film is soluble in concentrated formic acid. Weight loss of crosslinked corn starch films in formic acid was measured to see the crosslinking effect on acid stability. The weight loss of crosslinked starch films decreased significantly after crosslinking. Additionally, increasing crosslinking agent concentration increased the stability in acid. Non-crosslinked films were totally dissolved after being in formic acid for 24 hours as expected. However, even 1% crosslinked films were not dissolved as seen in Figure 21. Films crosslinked with 1% oxidized sucrose for 24 hours at  $25^{\circ}\text{C}$  have weight loss of 48.9%, which is still higher. Films crosslinked with 5% oxidized sucrose have weight loss of 25.9% and films crosslinked with 8%, 10% and 15% oxidized sucrose have weight loss of 19.4%, 18.7% and 17.5%, respectively, when treated in formic acid at  $25^{\circ}\text{C}$  for 24 hours. Films crosslinked with higher oxidized sucrose showed significant decrease in weight loss in formic acid. Crosslinking creates three dimensional compact polymeric structures. Thus, it provides better acid stability.

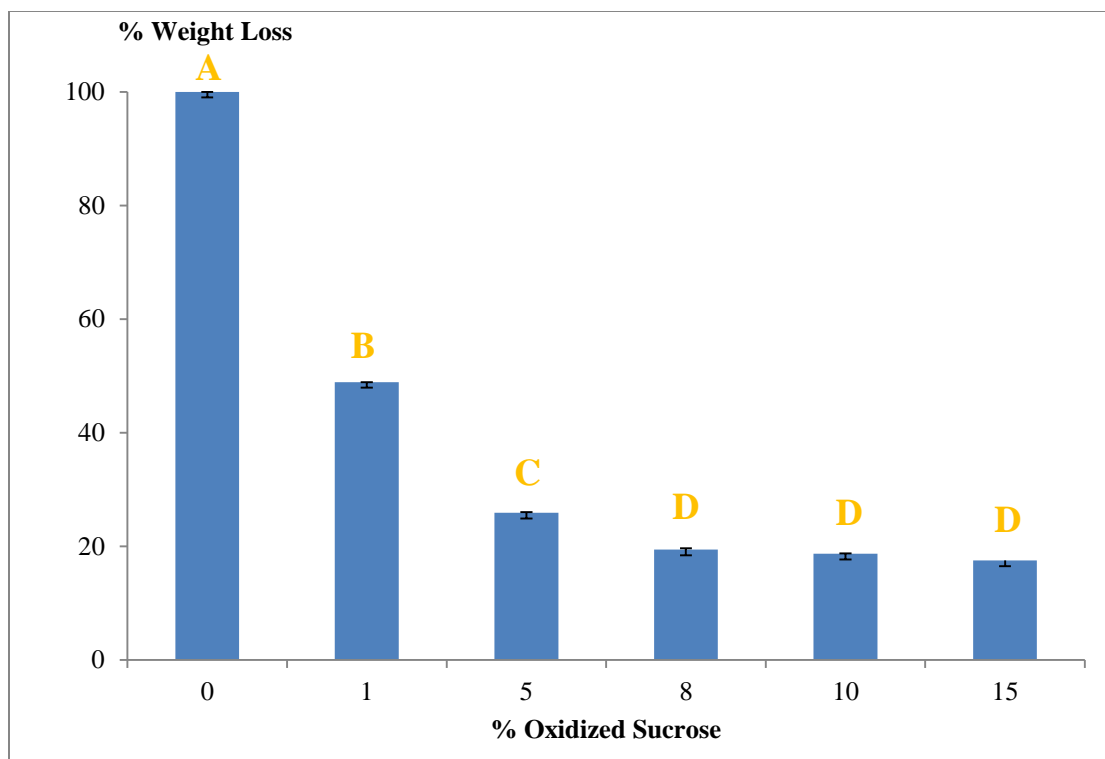


Figure 22. Weight loss of non-crosslinked and crosslinked starch films at 25°C in formic acid. Films were with 15% glycerol and cured at 165°C for 5 minutes.

## 5.7 Thermal Analysis

DSC is the technique used to determine the quantity of heat either absorbed or released when materials expose physical or chemical changes. The DSC thermograms of the non-crosslinked films and crosslinked corn starch films with 5% oxidized sucrose are shown in Figure 22. In non-crosslinked starch film thermogram, there is an endothermic peak that doubled around 160°C. The same peak at 160°C appeared in crosslinked starch film's thermogram, but it was unsharpened this time. Additionally, the second peak

shifted to higher temperature (188°C) after crosslinking. All these results show us, the first peak around 160°C shows the decomposition of starch molecules. For the non-crosslinked thermogram, the melting peak is lost in broad decomposition peak (this was explained as melting with decomposition by van Soest and Borger, 1997). On the other hand, after crosslinking, the melting peak shifted to higher temperature as stated earlier. This shows us the additional intra and intermolecular bonds formed by crosslinking agents increase the melting temperature, since they form more compact and stronger structure.

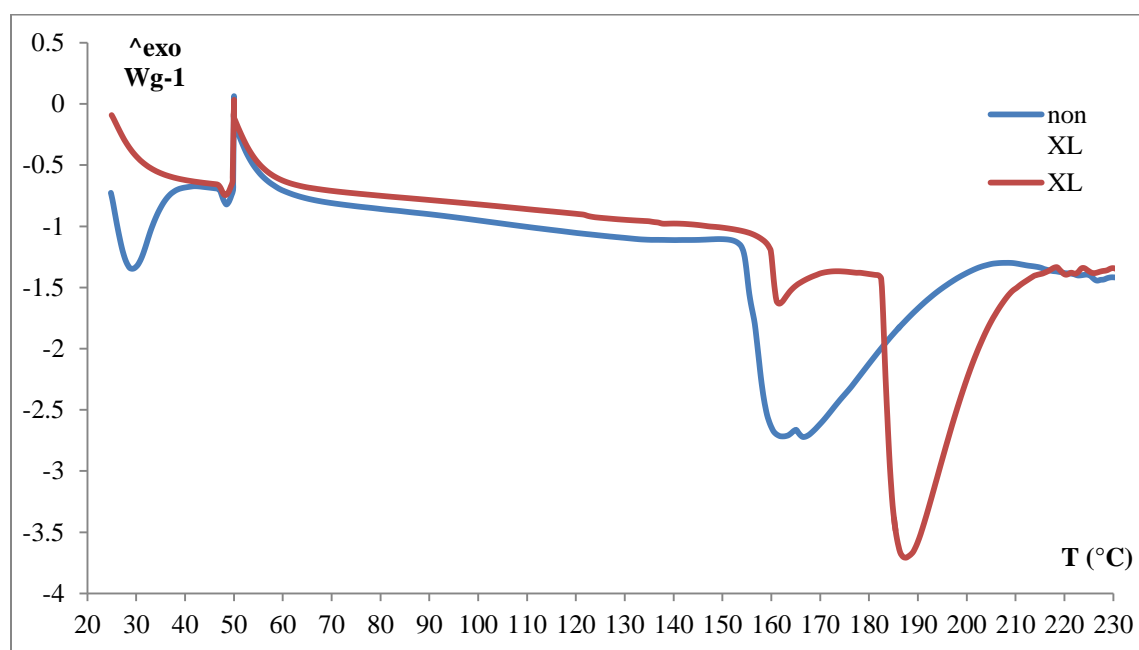


Figure 23. DSC thermograms of the non-crosslinked and crosslinked (5% oxidized sucrose) corn starch films

## 5.8 Reaction Kinetics of Oxidized Sucrose Crosslinked Starch Films

The crosslinking reaction of starch is a bimolecular reaction. In other words, it has mainly two components, oxidized sucrose (OS) and starch (St), as indicated in Figure 11 earlier. Thus, the rate of crosslinking reaction can be described as seen in equation (5), where  $[OS]$  is the oxidized sucrose concentration,  $[St]$  is the starch concentration,  $r$  is the reaction rate,  $k$  is the rate constant,  $a$  and  $b$  are the orders of reaction. To calculate the  $E_a$  and  $A$  values, rate order and rate constant should be determined.

$$r = -\frac{d[OS]}{dt} = -\frac{d[St]}{dt} = -k[OS]^a[St]^b \quad (5)$$

Since the hydroxyl groups of starch can be considered to be in large excess, the change in their concentration considered as negligible. Thus, it was expected that the reaction of aldehyde sides of oxidized sucrose with these hydroxyl groups follows pseudo-first-order kinetics (Lund, Brelid, 2013 and Schramm, Rinderer, 1997). A multiple linear regression technique was employed with the kinetic data set to determine the order of the reaction and the reaction rate was found as first order based on the standard error of the predicted y-value for each x in the regression. Thus, in the pseudo-first order rate equation,  $a$  will be 1, whereas  $b$  will be 0 based on the assumption made above. The pseudo-first order reaction rate was found as seen in equation (6), where  $k'$  is the new, pseudo-first order rate constant.

$$r = -\frac{d[OS]}{dt} = -k'[OS]^a \quad (6)$$

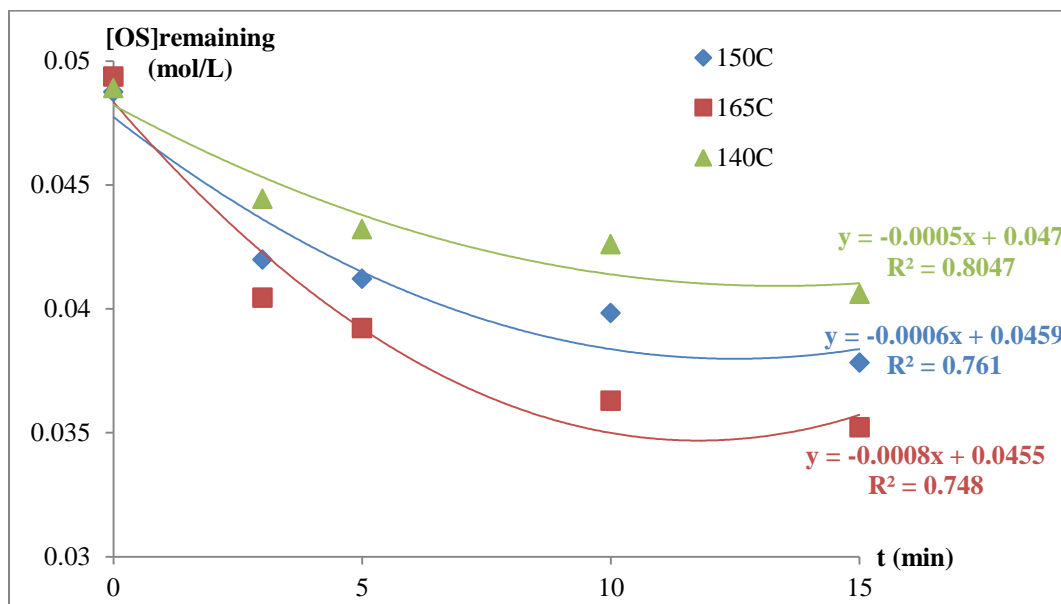


Figure 24. Decrease of the concentration of oxidized sucrose in the titration solution as a function of curing time for different curing temperatures (140°C, 150°C and 165°C). All films contained 5% oxidized sucrose.

After integration of the rate equation above, the correlation between oxidized sucrose and curing time was found as seen in equation (7), where  $[OS]_0$  indicates the concentration of oxidized sucrose at  $t=0$  minutes.

$$\ln[OS]_0 - \ln[OS] = k't \quad (7)$$



According to the equation (7), the rate constant,  $k'$ , can be determined from the value of the slope of the plot  $\ln [OS]_0 - \ln [OS]$  against the curing time,  $t$  and the regression lines were shown in Figure 24.

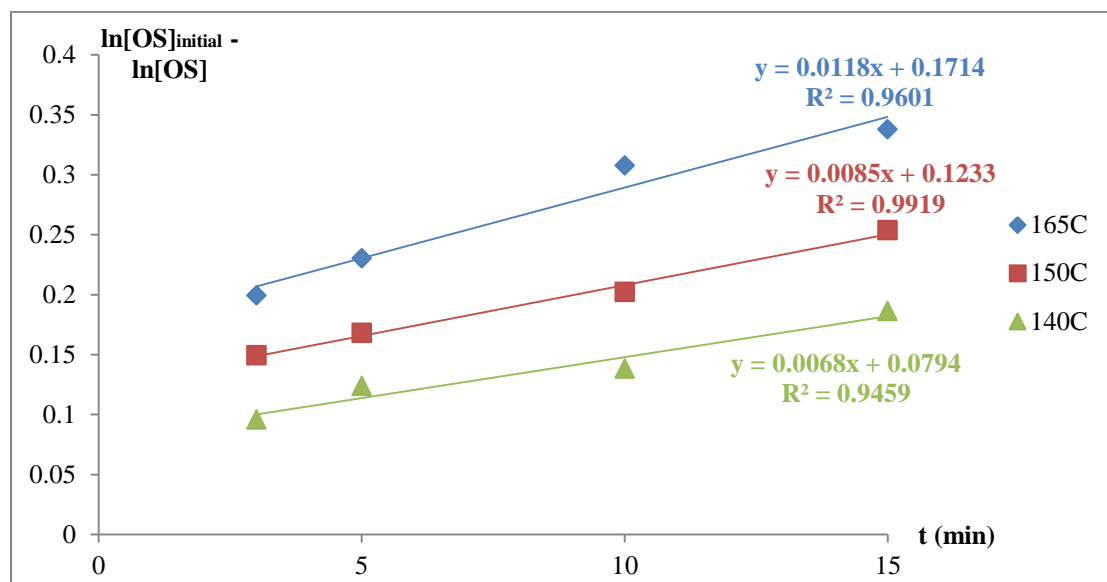


Figure 25. Regression lines to determine the reaction constants of oxidized sucrose at different curing temperatures (140°C, 150°C and 165°C).

After determining the rate constants for different curing temperatures,  $E_a$  was calculated from the slope of the plot  $\ln k$  against  $1/T$  and  $A$  was calculated from the intercept of the plot  $\ln k$  against  $1/T$  based on the equation (1) and the regression line can be seen in Figure 25.

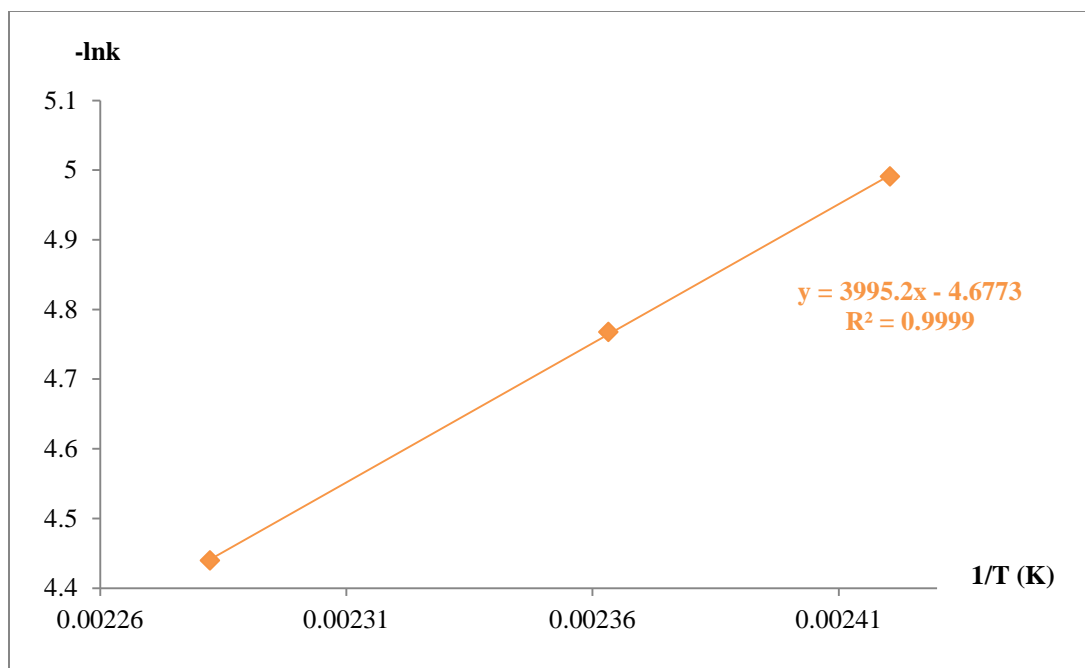


Figure 26. Regression line to determine the Arrhenius constant and Activation Energy of corn starch crosslinking with 5% oxidized sucrose.

Table 1. Kinetic data of the crosslinking reaction of 5% oxidized sucrose with corn starch at different curing temperatures

k (s <sup>-1</sup> x 10 <sup>-3</sup> )			E <sub>a</sub> (kJ/mol)	A (s <sup>-1</sup> )
140°C	150°C	165°C		
6.8	8.5	11.8	33.22	107.48

All these kinetic study results were shown in table 1. As it was expected, rate constant increased with curing temperature and the highest rate constant,  $11.8 \times 10^{-3} \text{ s}^{-1}$ , was obtained as a result of curing at higher temperature,  $165^\circ\text{C}$ . According to the Arrhenius equation stated in equation (1), rate constant and temperature has a negative correlation. In the molecular level, increase in the curing temperature will allow the molecules move faster and this will enhance the chance of reaction between the aldehyde groups of oxidized sucrose and hydroxyl groups of starch. The rate constant of crosslinking was  $6.8 \times 10^{-3} \text{ s}^{-1}$ , when the curing temperature was  $140^\circ\text{C}$ . It increased to  $11.8 \times 10^{-3} \text{ s}^{-1}$  when curing temperature increased to  $165^\circ\text{C}$ . Additionally, the Arrhenius constant was found as  $107.48 \text{ s}^{-1}$ .

On the other hand, the activation energy of the reaction, which indicates the minimum energy required to start crosslinking formation, was found  $33.22 \text{ kJ/mole}$ . This means, crosslinking between aldehyde groups of oxidized sucrose and hydroxyl groups of starch starts, when the energy in the system is only  $33.22 \text{ kJ/mole}$ , which is significantly lower than the activation energies of current crosslinking agents. Since polysaccharide structure is a complicated topic and it is tough to measure the degree of crosslinking of polysaccharides with high molecular weight, such as starch and cellulose, there is a lack of study on crosslinking kinetics. The comparison of oxidized sucrose with the current crosslinking agents can be seen in table 2 in terms of activation energy.

Table 2. Comparative values of activation energy for starch crosslinking with different crosslinkers

Source	Crosslinking Agent	$E_a$ (kJ/mole)	Author
<b>Corn</b>	Ammonium Zirconium Carbonate (AZC)	139	Delong, 2011
<b>Corn</b>	Epichlorohydrin (EPI)	38	Dumitriu, 2001
<b>Cassava</b>	Tetraethylene Glycol Diacrylate (TGD)	240	Marques et al., 2006
<b>Potato</b>	1, 2, 3, 4-diepoxbutane (DEB)	55	Simkovic et al., 2004
<b>Potato</b>	1, 2, 7, 8 – diepoxyoctane (DEO)	105	Simkovic et al., 2004

As seen in the comparison table, the lowest activation energy for starch crosslinking was obtained by EPI for corn starch, which was 38kJ/mole. In addition to the fact that the required activation energy is still lower than this value, EPI is classified as a human carcinogen as stated earlier. Additionally, previous researches resulted that DEB crosslinking requires 55kJ/mole for potato starch. Rather than these two crosslinkers, all the other agents have either high activation energies or not been studied yet. These comparisons show that, under the current circumstances, crosslinking reaction of starch with oxidized sucrose has the lowest activation energy. In other words, it is easier to crosslink with starch.

## CHAPTER 6: CONCLUSIONS

The claims of environmental safety and the lack of petroleum based materials provide a new opportunity for developing bio-based materials from the natural sources. A crosslinking agent was developed to obtain biodegradable corn starch films with high tensile strength, high elongation and high water stability.

The mechanical properties of corn starch films showed promising increases after crosslinking with oxidized sucrose. Crosslinked starch films that cured at 165°C for 5 minutes gave the best strength, 22.9 MPa and elongation, 59.5% values.

In addition to the increase in mechanical properties, the crosslinked films showed better stability either in distilled water or formic acid. Even after 35 days in water at 25°C, crosslinked films had only 23% weight loss. Also, crosslinked films had 25.9% weight loss after being in formic acid for 24 hours, whereas the non-crosslinked films were totally dissolved.

Last but not least, the developed titration method showed that the crosslinking reaction between starch and oxidized sucrose follows a pseudo-first order reaction, which depends on only oxidized sucrose and the activation energy of this crosslinking reaction is 33.22kJ/mole.

Thus, it can easily be considered as a sizing agent for paper-making industry. Oxidized sucrose crosslinked corn starch films have high strength, high elongation and high stability and the crosslinking reaction can be completed with small amount of energy.

## CHAPTER 7: REFERENCES

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